Journal of Medical Virology 56:316–320 (1998)

Influence of HIV-1 Infection on GBV-C Infection in Multiply Infected Haemophilic Patients

H. Devereux,[1]* C.A. Sabin,[2] Z. Kinson,[3] D. Brown,[4] A. Griffioen,[3] G.M. Dusheiko,[4] and C.A. Lee[3]

[1]*Department of Retrovirology, Royal Free Hospital, London, England*
[2]*HIV Research Unit, Department of Primary Care and Population Sciences, Royal Free Hospital, London, England*
[3]*Haemophilia Centre and Haemostasis Unit, Royal Free Hospital, London, England*
[4]*Department of Medicine, Royal Free Hospital, London, England*

Two hundred thirteen haemophilic patients were studied for the presence of GBV-C RNA and anti-E2 antibodies soon after their first treatment with unsterilised factor concentrates and in their most recent sample. An assessment was made to determine whether coinfection with HIV had any effect on the progression of GBV-C infection. All of the patients were infected with HCV and 81 patients (37%) were also infected with HIV. GBV-C RNA was detected using the Abbott LCx™ assay and by RT-PCR. Anti-E2 antibodies were detected using the μPLATE Anti-HGenv assay and by Abbott Laboratories. The HIV-negative patients were more likely than the HIV-positive patients to lose GBV-C RNA between the two time points. A proportion of the patients lost their anti-E2 antibodies over the time period, however, the majority of these were HIV-positive. This study shows that infection with HIV does affect the progression of GBV-C infection, however, this effect is little understood as yet. ***J. Med. Virol. 56:316–320, 1998.***

INTRODUCTION

All haemophilic patients treated with multidonor unsterilised blood products prior to 1985 were exposed to many viral pathogens, including hepatitis C virus (HCV) [Craske et al., 1978] and human immunodeficiency virus (HIV) [Goedert et al., 1985]. As a result of this, many of these patients have become chronically infected with HCV, and a significant proportion have also become chronically infected with HIV.

GBV-C/HGV has been recently identified [Simons et al., 1995; Linnen et al., 1996] from the plasma of a patient with etiologically unexplained chronic hepatitis (non–A-E hepatitis). GBV-C is a novel flavivirus with a single-stranded genome of positive sense RNA of approximately 9,400 nucleotides in length [Leary et al., 1996]. GBV-C is transmitted via blood and blood products, sexually and vertically [Simons et al., 1995; Feucht et al., 1996; Linnen et al., 1996; Stark et al., 1996].

Reported GBV-C incidence rates among haemophilic patients are approximately 14–38% [Jarvis et al., 1996; Linnen et al., 1996; Feucht et al., 1997]. This lower prevalence of GBV-C compared to HCV is not fully understood but one possibility is that GBV-C may be spontaneously cleared and that this usually coincides with the appearance of antibodies to the E2 protein of GBV-C [Pilot-Matias et al., 1996; Surowy et al., 1997; Tacke et al., 1997]. It is not yet established whether anti-E2 antibodies are neutralising, but it seems possible that they are a marker of recovery from infection.

The clinical relevance of GBV-C is unclear. Investigations into this subject have been complicated by the fact that the majority of patients infected with GBV-C also have other hepatotropic viruses such as hepatitis B and C viruses present. However, a recent study has shown that GBV-C may alter histological disease in patients with chronic HCV-related liver disease [Manolakopoulos et al., 1998]. The study of GBV-C is further complicated in haemophilic patients by the presence of HIV. The interaction of these viruses, and their clinical effect on the patient, is little understood at present.

Previous studies have looked at the effect of GBV-C on HIV infection [Toyoda et al., 1998] and also suggested that investigations into the prevalence of GBV-C infection require both antibody and nucleic acid detection [Gutierrez et al., 1997]. We have investigated the incidence of GBV-C RNA and anti-E2 antibodies at two time points in our population registered at the Haemophilia Centre and Haemostasis Unit, Royal Free Hospital, London, and assessed if infection with HIV had any effect on the course of GBV-C infection.

MATERIALS AND METHODS

Patients

There are 255 patients registered at the Haemophilia Centre and Haemostasis Unit at the Royal Free

*Correspondence to: Dr. H. Devereux, Department of Retrovirology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, England. E-mail: devereux@rfhsm.ac.uk

Accepted 10 June 1998

Hospital who have been infected with hepatitis C virus as a result of treatment with multidonor unsterilised clotting factor concentrates [Telfer et al., 1994]. In 1979, a serum store was established in the department and every patient has had at least one aliquot of serum stored at every clinic visit since then.

Two hundred and thirteen patients had sufficient serum stored for this study. Their diagnoses were 156 haemophilia A, 33 haemophilia B, 16 von Willebrand's disease, and 8 with other disorders or in whom the disorder was not documented. All patients were hepatitis C antibody-positive and 82 patients were HIV antibody-positive.

The carriage rate of GBV-C in blood donors in northern Europe is much higher than that of HCV (1–3% [Dawson et al., 1996; Linnen et al., 1996] and 0.1–0.5% [van der Poel et al., 1994], respectively). The majority of haemophilic patients treated with unsterilised clotting factor concentrates have become anti-HCV–positive [Makris et al., 1990]. With a blood donor population carriage rate of GBV-C significantly higher than that of HCV, haemophilic patients could be assumed to have been exposed to GBV-C at the time of their first treatment with unsterilised blood products. For this reason we tested one sample soon after the date of first exposure (early sample) and a second most recent sample from each patient in the study. For those in whom the date of first exposure was unknown, we tested the first available sample. In patients known to have died, the most recent sample prior to death was tested. The median time between these two samples was 10.7 years (range 2 months to 17 years).

GBV-C RNA Detection

For 97 HCV antibody-positive, HIV antibody-negative and 69 HCV-positive, HIV-positive patients, the early and recent samples were tested for GBV-C using the Abbott LCx™ assay (equipment and reagents kindly provided by Dr. Thomas Laffler, Abbott Laboratories, Chicago, IL) according to the manufacturer's instructions. The remainder of the patients' early and recent samples were tested using GBV-C PCR with primers in the 5′UTR of GBV-C [Jarvis et al., 1996].

GBV-C RNA was extracted from serum samples stored at –40°C using the Qiagen QIAamp Viral RNA Kit according to manufacturer's instructions. The extraction product was heated to 70°C for 5 min immediately prior to reverse transcription. The GBV-C RNA was reverse-transcribed to cDNA in a volume of 25 μl containing 10 μl of template, 25 pmoles of random hexamers (Pharmacia, Piscataway, NJ), 2.5 μl of 0.5-mM dNTPs (Boehringer Mannheim, UK), 250 units of Moloney-Murine Leukaemia Virus (M-MLV) reverse transcriptase (GIBCO-BRL, Bethesda, MD), and 5 μl of 5 × First Strand Buffer (250-mM Tris-HCl pH 8.3, 375-mM KCl, 15-mM $MgCl_2$). The reaction was incubated for 1 hr at 37°C. The cDNA product was heated to 95°C for 5 min prior to amplification.

The GBV-C cDNA was amplified using two rounds of PCR (nested). The reaction mixture for the first round contained 5-μl template, 16-μl 1.25-mM dNTPs, 50 pmoles of each primer (Oswell DNA Services, UK), 5 units of Taq polymerase (Bioline, UK), 3 μl of 50-mM $MgCl_2$ (Bioline), and 10 μl of 10 × Taq buffer [160-mM $(NH_4)_2SO_4$, 670-mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20] in a final volume of 100 μl. The second-round PCR mixture contained 2 μl of the first-round product, 8-μl 1.25-mM dNTPs, 50 pmoles of each primer, 5 units of Taq polymerase, 1.5 μl of 50-mM $MgCl_2$ and 5 μl of Taq buffer in a final volume of 50 μl.

The sequence of the outer primers was 5′-AGGTGGTGGATGGGTGAT-3′ and 5′-TGCCACCCGCCCTCACCCGAA-3′, and the inner primers were 5′-TGGTAGGTCGTAAATCCCGGT-3′ and 5′-GGRGCTGGGTGGCCYCATGCWT-3′. The first-round PCR conditions were 5 cycles at 94°C for 1.5 min, 50°C for 2 min, and 72°C for 3 min followed by 30 cycles at 94°C for 1.5 min, 60°C for 1.5 min, and 72°C for 2 min, with a final extension of 72°C for 5 min. The second-round PCR conditions were 30 cycles at 94°C for 1 min and 24 sec, 40°C for 1 min, and 72°C for 1 min with a final extension of 72°C for 5 min. The PCR product (343 bases) was visualised on a 2% agarose gel using ethidium bromide.

Anti-E2 Antibody Measurements

The early and the recent samples for the 97 HCV antibody-positive, HIV antibody-negative patients tested for GBV-C RNA using the Abbott LCx™ assay were tested for anti-HGenv by Abbott Laboratories. The remainder of the patients' (116) early and recent samples was tested using the μPLATE Anti-HGenv assay (Boehringer Mannheim) according to the manufacturer's instructions.

Statistical Methods

Differences in the prevalence of GBV-C RNA and anti-E2 antibodies between HIV-positive and HIV-negative individuals were tested for significance using Fisher's exact test. Univariate and multivariate relationships between detection of GBV-C RNA and anti-E2 antibodies at each time point and three factors of interest (age of patient, time since first exposure to clotting factor concentrates, and HIV status) were investigated using univariate and multivariate logistic regression methods. All statistical analyses were performed using the statistical analysis system (SAS).

RESULTS

None of the 213 patients included in this study was found with GBV-C RNA and anti-E2 antibodies in the same sample (early or recent). Thirty-four patients (16%) had no evidence of infection at either time point (both samples were GBV-C RNA– and anti-E2–negative). For the early sample, 33 patients (16%) were GBV-C RNA–positive and 129 patients (61%) were anti-E2–positive; whereas for the recent sample, 23 patients (11%) were GBV-C RNA–positive and 117 were

TABLE I. GBV-C/HGV RNA and Antibodies to GBV-C/HGV E2 Glycoprotein in 213 Patients at Two Time Points

	Early sample	Recent sample
HIV–		
HGV RNA+	23 (18%)	12 (9%)
Anti-E2 ab+	82 (63%)	92 (70%)
HIV+		
HGV RNA+	10 (12%)	11 (13%)
Anti-E2 ab+	47 (57%)	25 (30%)

anti-E2–positive (55%) (see Table I). When the patients were divided into two groups according to their HIV status, 18% of HIV-negative patients and 12% of HIV-positive patients were GBV-C RNA–positive for their early sample and 9% and 13%, respectively, were positive in their recent sample.

Patients were then separated into nine groups (0–8) according to their GBV-C RNA and anti-E2 antibody results on the early and recent samples (see Table II). These results show that the majority of HIV-negative patients, 75/131 (57%), were exposed to GBV-C before their early sample was taken, which resulted in a persistent anti-E2 response and they did not become chronically infected with GBV-C (group 8). The other HIV-negative patients were distributed relatively evenly across the groups, with no patients showing any evidence for infection after anti-E2 became detectable (group 7) and five patients (4%) who lost GBV-C RNA without detectable anti-E2 antibody (group 3).

The HIV-positive patients showed a significantly different distribution between the groups ($P = 0.001$, Fisher's exact test). Nineteen patients (23%) showed no evidence of exposure to GBV-C (group 0) and a further 22 (27%) patients had a persistent anti-E2 antibody response with no detectable GBV-C RNA at either time point (group 8). The largest number of patients, 24 (29%), were exposed before the early sample and had a detectable anti-E2 response in the early sample and subsequently lost the anti-E2 antibody by the recent sample (group 6). The remainder of the HIV-positive patients were distributed relatively evenly between the other groups, with one patient (1%) becoming GBV-C RNA–positive after anti-E2 became detectable (group 7) and three patients (4%) who lost GBV-C RNA without detectable anti-E2 antibody (group 3).

Investigations were made to consider whether there was an association between detection of GBV-C RNA or anti-E2 antibody at either time point and either the age of the patient at the time of the sample, the time of the sample since first exposure to clotting factor, or the patient's HIV status. None of the factors were significantly associated with PCR detection at either time point. However, older patients were more likely to have detectable anti-E2 antibodies at both time points [early sample: odds ratio (OR) for five-year difference in age 1.24, 95% confidence interval (CI) 1.11–1.39, $P = 0.0002$; late sample: OR for five year difference in age 1.28, 95% CI 1.14–1.43, $P = 0.0001$]. Individuals with detectable anti-E2 antibodies at the time of the early sample tended to have been exposed to clotting factor concentrates for longer periods (OR for five year difference in time since exposure 1.68, 95% CI 1.06–2.68, $P = 0.03$), although this became nonsignificant after adjusting for the age of the patient. HIV-positive individuals were less likely to have detectable anti-E2 antibodies at the recent sample (OR 0.19, 95% CI 0.10–0.34, $P = 0.0001$). This effect was independent of the age of the patient.

DISCUSSION

In this large clinic cohort of individuals with haemophilia, it was shown that those infected with HIV are more likely to lose detectable antibodies to the E2 protein of GBV-C over time than those uninfected with HIV. Whilst other studies [Toyoda et al., 1998] have assessed the effect of GBV-C on HIV infection in small numbers of patients, none have looked at the effect of coinfection with HIV on the course of GBV-C infection in a substantial number of well-characterized HIV-positive patients utilising both GBV-C RNA and anti-E2 antibody measurements over a median of 10 years.

The prevalence of GBV-C RNA in this population was within the range reported by previous studies [Jarvis et al., 1996; Linnen et al., 1996; Feucht et al., 1997], with the early sample having a slightly higher prevalence than the recent sample. This is probably explained by clearance of the virus over time. A previous study has also shown that GBV-C may be cleared early in infection as the patients in this study infected with GBV-C and HCV were younger than those infected with HCV alone [Manolakopoulos et al., 1998]. However, this trend was not seen in the HIV-positive patient group. The HIV-negative patients were more likely to lose GBV-C RNA between the two time points, whereas the HIV-positive patients were more likely to gain GBV-C RNA. This suggests that the HIV-negative patients were more successful at clearing GBV-C and that the HIV-positive patients may have been infected or reinfected after their early sample and were unable to clear this infection.

One HIV-positive patient was anti-E2 antibody–positive in his early sample and GBV-C RNA–positive in his recent sample (group 7). This could be due to a loss of the anti-E2 antibody and a subsequent reinfection with GBV-C prior to the second time point. Longitudinal GBV-C PCR in this patient showed that reinfection occurred prior to the introduction of sterilised clotting factor concentrates in 1985.

Interestingly, older patients in our cohort were more likely to have detectable antibodies at either time point than younger individuals, independently of the HIV status of the patient or the length of time exposed to clotting factor concentrates.

HIV coinfection with HCV can increase the progression to liver failure and AIDS [Eyster et al., 1993]. In this study, we have shown that infection with HIV does affect the course of GBV-C infection. It is now well established that HIV infection usually results in a severe depression of the immune system [Lane et al.,

TABLE II. GBV-C/HGV RNA and Antibodies to GBV-C/HGV E2 Glycoprotein Results

Group	Early sample PCR	Early sample Anti-E2	Recent sample PCR	Recent sample Anti-E2	HIV– patients, n = 131	HIV+ patients, n = 82
0 No exposure detected	–	–	–	–	15 (12%)	19 (23%)
1 Exposure between samples, chronic infection	–	–	+	–	2 (2%)	4 (5%)
2 Exposure between samples, antibody response, no infection	–	–	–	+	9 (7%)	2 (2%)
3 Loss of infection without antibody	+	–	–	–	5 (4%)	3 (4%)
4 Exposure before early sample, chronic infection	+	–	+	–	10 (8%)	6 (7%)
5 Exposure before early sample, antibody response, no infection	+	–	–	+	8 (6%)	1 (1%)
6 Exposure before early sample, antibody response, loss of antibody	–	+	–	–	7 (5%)	24 (29%)
7 Infection after antibody response	–	+	+	–	0	1 (1%)
8 Exposure before early sample, antibody response, no infection	–	+	–	+	75 (57%)	22 (27%)

1985]. These data show that HIV-positive patients were less likely to clear their GBV-C infection than HIV-negative patients. When anti-E2 antibodies are produced, they appear to be lost with a significantly higher frequency in HIV-positive patients than in HIV-negative patients. Possibly as a result of this loss, one HIV-positive patient appears to have been reinfected with GBV-C. This suggests that the presence of anti-E2 antibody may have a protective effect against GBV-C.

This study demonstrates that infection with HIV seems to increase the possibility of chronic GBV-C infection and a reduced number of anti-E2 antibodies, which leaves these patients susceptible to reinfection with GBV-C.

The clinical significance of GBV-C infection has not been clarified; however, coinfection with GBV-C, HCV, and HIV may result in complex viral interactions, which may cause clinical complications that are not fully understood as yet.

ACKNOWLEDGMENTS

Z.K. was supported by the Katharine Dormandy Trust.

REFERENCES

Craske J, Spooner RJD, Vandervelde EM (1978): Evidence for existence of at least two types of factor-VIII-associated non-B transfusion hepatitis. Lancet ii:1051–1052.

Dawson GJ, Schlauder GG, Pilot-Matias TJ, Thiele D, Leary TP, Murphy P, Rosenblatt JE, Simons JN, Martinson FE, Gutierrez RA, Lentino JR, Pachucki C, Muerhoff AS, Widell A, Tegtmeier G, Desai S, Mushahwar IK (1996): Prevalence studies of GB virus-C infection using reverse transcriptase–polymerase chain reaction. Journal of Medical Virology 50:97–103.

Eyster ME, Diamondstone LS, Lien JM, Ehmann WC, Quan S, Goedert JJ (1993): Natural history of hepatitis C virus infection in multitransfused hemophiliacs : Effect of coinfection with human immunodeficiency virus: The Multicentre Haemophilia Cohort Study. Journal of Acquired Immune Deficiency Syndrome 6:602–610.

Feucht H, Zollner B, Polywka S, Laufs R (1996): Vertical transmission of hepatitis G virus. Lancet 347:615.

Feucht H, Zollner B, Polywka S, Knodler B, Schroter M, Nolte H, Laufs R (1997): Prevalence of hepatitis G viraemia among healthy subjects, individuals with liver disease, and persons at risk for parenteral transmission. Journal of Clinical Microbiology 35:767–768.

Goedert JJ, Sarngadharan MG, Eyster ME, Weiss SH, Bodner AJ, Gallo RC, Blattner WA (1985): Antibodies reactive with human T cell leukaemia viruses in the serum of hemophiliacs receiving factor VIII concentrate. Blood 65:492–495.

Gutierrez RA, Dawson GJ, Knigge MF, Melvin SL, Heynen CA, Kyrk CR, Young CE, Carrick RJ, Schlauder GG, Surowy TK, Dille BJ, Coleman PF, Thiele DL, Lentino JR, Pachucki C, Mushahwar IK (1997): Seroprevalence of GB virus C and persistence of RNA and antibody. Journal of Medical Virology 53:167–173.

Jarvis LM, Davidson F, Hanley PL, Ludlam CA, Simmonds P (1996): Infection with hepatitis G virus among recipients of plasma products. Lancet 348:1352–1355.

Lane HC, Masur H, Gelmann EP, Longo DL, Steis RG, Chused T, Whalen G, Edgar LC, Fauci AS (1985): Correlation between immunologic and clinical subpopulations of patients with the acquired immune deficiency syndrome. American Journal of Medicine 78:417–422.

Leary TP, Muerhoff AS, Simons JN, Pilot-Matias TJ, Erker JC, Chalmers ML, Schlauder GG, Dawson GJ, Desai SM, Mushahwar IK (1996): Sequence and genomic organisation of GBV-C: A novel member of the *Flaviviridae* associated with human non A-E hepatitis. Journal of Medical Virology 48:60–67.

Linnen J, Wages J, Zhang-Keck Z-Y, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Wai-Kuo Shih J, Young L, Piatak M Jr., Hoover C, Fernandez J, Chen S, Zou J-C, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Thomas H, Bradley D, Margolis H, Kim JP (1996): Molecular cloning and disease association of hepatitis G virus: A transfusion-transmissible agent. Science 271:505–508.

Makris M, Preston FE, Triger DR, Underwood JCE, Choo Q-L, Kuo G, Houghton M (1990): Hepatitis C antibody and chronic liver disease in haemophilia. Lancet 335:1117–1120.

Manolakopoulos S, Morris A, Davies S, Brown D, Hajat S, Dusheiko G (1998): Influence of GB virus C viraemia on the clinical, virological and histological features of early hepatitis C-related hepatic disease. Journal of Hepatology 28:173–178.

Pilot-Matias TJ, Carrick RJ, Coleman PF, Leary TP, Surowy TK, Simons JN, Muerhoff AS, Buijk SL, Chalmers ML, Dawson GJ, Desai SM, Mushahwar IK (1996): Expression of the GB virus C E2 glycoprotein using the Semliki Forest virus vector system and its utility as a serological marker. Virology 225:282–292.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK (1995): Isolation of novel virus-like sequences associated with human hepatitis. Nature Medicine 1:564–569.

Stark K, Bienzle U, Hess G, Engel AM, Hegenscheid B, Scluter W (1996): Detection of the hepatitis G virus genome among injecting drug users, homosexual and bisexual men and blood donors. Journal of Infectious Diseases 174:1320–1323.

Surowy TK, Leary TP, Carrick RJ, Knigge MF, Pilot-Matias TJ, Hey-

nen C, Gutierrez RA, Desai SM, Dawson GJ, Mushahwar IK (1997): GB virus C E2 glycoprotein: Expression in CHO cells, purification and characterization. Journal of General Virology 78: 1851–1859.

Tacke M, Kiyosawa K, Stark K, Schlueter V, Ofenloch-Haehnle B, Hess G (1997): Detection of antibodies to a putative hepatitis G virus envelope protein. Lancet 349:318–320.

Telfer PT, Sabin C, Devereux HL, Scott F, Dusheiko GM, Lee CA (1994): The progression of HCV-associated liver disease in a cohort of haemophilic patients. British Journal of Hematology 87:555–561.

Toyoda H, Fukuda Y, Hayakawa T, Takamatsu J, Saito H (1998): Effect of GB virus C/hepatitis G virus coinfection on the course of HIV infection in hemophilia patients in Japan. Journal of Acquired Immune Deficiency Syndrome and Human Retrovirology 17:209–213.

van der Poel CL, Cuypers HT, Reesink HW (1994): Hepatitis C virus six years on. Lancet 344:1475–1479.